

We Claim:

1. A kit for amplifying HCV nucleic acid comprising:

a first amplification primer having the sequence:

5'-gcagaaaagcgtagccatggcgt-3' [SEQ. ID. NO. 1]

and a second amplification primer having the sequence:

5'-ctcgcaaggccatcaggcgt-3' [SEQ. ID. NO. 2]

2. The kit according to claim 1, wherein the first amplification primer comprises

at least twenty four continuous bases selected from the polyprotein gene sense strand.

3. The kit according to claim 1, wherein the first amplification primer is

present in an amount of about 10 to about 100 pM.

4. The kit according to claim 1, wherein the second amplification primer

comprises at least twenty four continuous bases selected from the polyprotein gene anti-sense strand.

5. The kit according to claim 1, wherein the second amplification primer is

present in an amount of about 10 to about 100 pM.

6. The kit according to claim 1, further comprising RNA dependent DNA

Polymerase.

7. The kit according to claim 6, wherein the RNA dependent DNA polymerase is Avian Mycloblastosis Virus present in an amount of about 5 units to about 10 units.

8. The kit according to claim 1, further comprising DNA dependent DNA polymerase.

9. The kit according to claim 8, wherein the DNA dependent DNA polymerase is Taq polymerase present in an amount of about 1 Unit to about 2.5 Units.

10. The kit according to claim 1, further comprising a deoxyribonucleoside triphosphates.

11. The kit according to claim 10, wherein said deoxyribonucleoside triphosphates are selected from the group consisting of dATP, dCTP, 5MedCTP, dGTP, dITP, TTP, dUTP, and combinations thereof.

12. The kit according to claim 11, wherein said deoxyribonucleoside triphosphate is present in an amount of about 100 to about 200 μ M.

13. The kit according to claim 1, wherein the first and second amplification primers have a label at their respective 5' ends.

14. The kit according to claim 13, wherein the label is fluorescein.

15. A kit for detecting an HCV nucleic acid comprising an oligonucleotide probe

having the sequence:

5'-gtcgtgcagcctccaggaccc-3' [SEQ. ID. NO. 3]

16. A kit according to claim 15, wherein the sequence of the oligonucleotide probe is internal to an amplimer resulting from the amplification using SEQ. ID. NO. 1 and SEQ. ID. NO. 2

17. A kit according to claim 15, wherein the oligonucleotide probe has a label at their 5' end.

18. A kit according to claim 17, wherein the label is biotin.

19. The kit according to claim 15, wherein the oligonucleotide probe is immobilized on a solid medium.

20. The kit according to claim 15, wherein the oligonucleotide probe is present in an amount of about 10 to about 100 pM.

21. The kit according to claim 15, further comprising a conjugate adapted to bind with a label present on the HCV nucleic acid.

22. The kit according to claim 21, wherein the selected label is fluorescein and the conjugate is an anti-fluorescein/horse raddish peroxidase conjugate present in an amount of about 1 Unit to about 4 Units.

23. The kit according to claim 21, further comprising a substrate adapted to change color in the presence of an enzyme on the conjugate.

24. The kit according to claim 23, wherein the detection solution comprises hydrogen peroxide and 3,3',5,5'-Tetra methyl benzidine Dihydrochloride.

25. The kit according to claim 24, wherein the substrate is present in an amount of about 100 μ L.

26. A method for detecting HCV nucleic acid in a biological sample comprising the steps of:

extracting HCV nucleic acid a biological sample;

active reverse transcription of the extracted nucleic acid using the reverse strand primer;

and

amplifying the HCV nucleic acid using a first primer having the sequence
5'-gcagaaaagcgcttagccatggcgt-3' [SEQ. ID. NO. 1]

and a second primer having the sequence

5'-ctcgcaagcaccctatcaggcagt-3' [SEQ. ID. NO. 2]

and detecting the HCV nucleic acid using an oligonucleotide probe having the sequence:

5'-gtcgfgcagcctccaggaccc-3' [SEQ. ID. NO. 3]

27. The method according to claim 26, wherein the biological sample is selected from the group consisting of: serum, plasma, and combinations thereof.

28. The method according to claim 26, wherein the first and second amplification primers have a label at their respective 5' ends.

29. The method according to claim 28, wherein the label is fluorescein.

30. The method according to claim 26, wherein the step of amplifying the HCV nucleic acid includes:

denaturing the HCV nucleic acid to produce denatured HCV nucleic acid;

annealing the first and second amplification primers to the denatured HCV nucleic acid to produce primed HCV nucleic acid; and

extending the primed HCV nucleic acid using a thermostable DNA dependent DNA polymerase in the presence of a deoxyribonucleoside triphosphate.

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31. The method according to claim 30, wherein the DNA dependent DNA polymerase is Taq polymerase present in an amount of about 1 Unit to about 2.5 Units.

32. The method according to claim 30, wherein the deoxyribonucleoside triphosphate is selected from the group consisting of: dATP, dCTP, 5MedCTP, dGTP, dITP, TTP, dUTP, and combinations thereof, and wherein the deoxyribonucleoside triphosphate is present in an amount of about 100 to about 200 μ M.

33. The method according to claim 26, wherein the step of detecting the HCV

nucleic acid includes:

binding the HCV nucleic acid with the oligonucleotide probe attached to a solid

medium to form immobilized HCV nucleic acid;

binding the immobilized HCV nucleic acid with a conjugate; and

adding a substrate that is adapted to change color in the presence of an enzyme on

the conjugate,

whereby a change of the color of the substrate indicates the presence of HCV

nucleic acid.

34. The method according to claim 33, wherein the HCV nucleic acid is labeled with fluorescein, and wherein the detectable marker is an anti-fluorescein/horse radish peroxidase conjugate in an amount of about 1 unit to about 4 units.

35. The method according to claim 33, wherein the substrate compromises

hydrogen peroxide and 3,3',5,5'-Tetra methyl benzidine Dihydrochloride.

36. The method according to claim 33, wherein the substrate is present in an

W B
amount of about 100 μ L.

37. The method according to claim 33, further comprising the step of reading a

add B
change of the color of the substrate with a colorimetric plate reader.